JOURNAL OF ANIMAL SCIENCE
The Premier Journal and Leading Source of New Knowledge and Perspective in Animal Science

Low-phytic acid corn improves nutrient utilization for growing pigs

T. L. Veum, D. R. Ledoux, V. Raboy and D. S. Ertl

J Anim Sci 2001. 79:2873-2880.

Low-phytic acid corn improves nutrient utilization for growing pigs[1]

T. L. Veum*[2], D. R. Ledoux*, V. Raboy†, and D. S. Ertl‡

*Department of Animal Sciences, University of Missouri, Columbia, MO 65211-5300; †National Small Grains Germplasm Research Facility, USDA-ARS, 1691 S. 2700 W., Aberdeen, ID 83210; and ‡Pioneer Hi-Bred International, Inc., 7301 N.W. 62nd Ave., Johnston, IA 50131-0085

ABSTRACT: Thirty-five crossbred barrows averaging 14.5 kg initial BW were used in a 5-wk experiment to compare the P availability and nutritional value of a low-phytate hybrid corn (LPC, 0.26% total P, 0.08% phytic acid P) homozygous for the *lpa 1-1* allele with a nearly isogenic normal hybrid corn (NC, 0.25% total P, 0.20% phytic acid P). The pigs were fed individually twice daily in metabolism pens. Three semipurified diets were created in which corn was the only source of phytate. Diet 1 contained 72% NC, 0.15% estimated available P (aP) and 0.55% Ca. Diet 2 contained 72% LPC, 0.24% aP, and 0.55% Ca. The only differences between Diets 1 and 2 were the source of corn and the levels of aP. No inorganic P (iP) was added to these diets in order to measure the animal response to the different levels of aP in the corn hybrids. Diet 3 was NC Diet 1 supplemented with iP to equal the level of aP in LPC Diet 2. Diets 4 and 5 were practical corn-soybean meal diets formulated with each corn to meet all minimum nutrient requirements and contained 0.30% aP and 0.65% Ca. For the semipurified diets, pigs fed LPC Diet 2 had higher ($P < 0.01$) growth performance, bone breaking strength, P absorption and retention, Ca absorption and retention, and N retention than pigs fed NC Diet 1. However, when the NC diet was supplemented with iP to equal the aP in the LPC diet, most criteria were similar ($P \geq 0.2$), indicating an equal nutritional value for both corn hybrids after adjusting for phytate level. The only treatment difference, other than P excretion, between the practical corn diets supplemented with soybean meal was a higher ($P < 0.05$) bone breaking strength for pigs fed LPC Diet 5 compared with NC Diet 4. The use of LPC in pig diets reduced P excretion in swine waste by 50 and 18.4% in the semipurified and practical diets, respectively, compared with NC. Using our in vitro procedure designed to simulate the digestive system of the pig, the availability of P for pigs was estimated at 56% for LPC and 11% for NC.

Key Words: Maize, Phosphorus, Phytic Acid, Pigs

 J. Anim. Sci. 2001. 79:2873–2880

Introduction

About 85% of the P in a normal corn-soybean meal diet fed to swine is not utilized because it is bound as phytate (myo-inositol 1,2,3,4,5,6-hexakis-dihydrogen phosphate; Maga, 1982; Lott et al., 2000). Swine lack the digestive enzyme phytase, which is needed to digest phytate (Pointillart et al., 1984, 1987). Supplementation of corn-soybean meal diets with a recombinant-derived microbial phytase (Heinzl, 1996) increases phytate hydrolysis and P utilization by swine, and reduces P excretion in swine waste (Cromwell et al., 1993, 1995; Liu et al., 1997a). The effectiveness of microbial phytase is affected by the dietary Ca:total P ratio (Liu et al., 1998a; 2000), and pelletizing or the extrusion of the diets may destroy phytase activity (Simons et al., 1990; Guenther, 1996).

The development of a low-phytic acid corn hybrid (**LPC**) by USDA scientists that has a much higher percentage of available P for nonruminants compared with a near-isogenic normal corn (NC) hybrid (Ertl et al., 1998; Sugiura et al., 1999; Li et al., 2000) is expected to reduce the excretion of P in swine waste. The objectives of this experiment were to confirm the increased availability of P in LPC and to determine whether the decrease in phytic acid content in LPC would change the nutritional value of LPC. In addition, an in vitro procedure that simulates the digestive system of the

[1]David Bollinger, Qiang Zhang, Jessica Smith, and LeAnn Harmon are acknowledged for their assistance in data collection and laboratory analysis.

[2]Correspondence: 112 Animal Science Center (phone: 573-882-4331; fax 573-882-6827; E-mail: veumt@missouri.edu).

Received November 3, 2000.

Accepted June 8, 2001.

growing pig was used to estimate the availability of P in the two corn hybrids.

Materials and Methods

In Vivo Experiment

Animals and Housing. Thirty-five crossbred (Yorkshire-Landrace-Duroc) barrows averaging 14.5 ± 0.1 kg and 42 ± 1 d of age were allotted to five treatments in a completely random design for the 35-d experiment. Pigs were placed in individual elevated stainless steel metabolism pens (20 pens with 0.9 m^2/pig and 15 pens with 0.7 m^2/pig) equipped with nipple drinkers, stainless steel feeders, and stainless steel woven wire floors. Temperature was maintained at 22 ± 1°C with thermostatically controlled heaters and exhaust fans. Pigs were fed to appetite twice daily (0730 and 1530). Individual feed consumption was determined weekly. Pigs were weighed at the beginning and end (d 0 and 35) of the test. This experiment was approved by the University of Missouri Animal Care and Use Committee.

Dietary Treatments. The LPC and NC hybrids used in this experiment were provided by DuPont Specialty Grains, Johnston, IA. The LPC and NC grains were produced by sibling, nearly isogenic corn hybrids. The LPC isohybrid was homozygous for the recessive low-phytic acid 1-1 allele of the low phytic acid 1 gene, and produced grain with about 60% less phytic acid than the NC. The NC hybrid was homozygous for the dominant, wild type allele of that gene and produced grain with normal concentrations of phytic acid. Before diet formulation, stocks of the mutant LPC hybrid, the NC hybrid, the soybean meal, the whey protein concentrate, and the spray-dried blood cells were analyzed in duplicate for proximate analysis components (AOAC, 1990) and complete amino acids according to Benson and Patterson (1971). Samples were hydrolyzed under N with 6 *N* HCl for 24 h at 110°C before amino acid analysis was performed by automated cation exchange chromatography. Analysis for cystine and methionine involved performic acid oxidation prior to hydrolysis. Tryptophan was determined by the method of Spies and Chambers (1949).

Triplicate samples of the above ingredients plus dicalcium phosphate, monosodium phosphate, and ground limestone were digested with a wet ash procedure (AOAC, 1990). Digests were analyzed for the concentration of total P (minus ground limestone) by the colorimetric molybdovanadate method (Spectra Rainbow Microplate Reader, Tecan, Inc., Durham, NC) and for the concentration of Ca (minus monosodium phosphate) by atomic absorption spectrophotometry (Spector AA-30, Varian Analytical Instruments, San Fernando, CA). Subsamples of both corn hybrids and soybean meal were analyzed for phytic acid as described by Raboy et al. (2000). The difference between total P and phytic acid P concentration was defined as calculated available P. The nutrient composition of the two corn hybrids (Table 1) was similar except for the differences in phytic acid P concentration and calculated available P.

Table 1. Analyzed composition (%) of air-dry corn hybrids[a]

	Corn hybrids	
Nutrient	Normal corn	Low-phytate mutant corn
Total P	0.25	0.26
Phytic acid P	0.20	0.08
Available P	0.05	0.18
Ca	0.01	0.01
Lysine	0.24	0.26
Methionine	0.15	0.16
Cysteine	0.19	0.20
Tryptophan	0.06	0.06
Threonine	0.29	0.29
Isoleucine	0.33	0.32
Valine	0.43	0.43
CP	8.80	8.60

[a]Analyzed values are based on duplicate or triplicate analyses determined prior to the formulation and mixing of the diets. Available P was calculated by subtracting phytic acid P from total P.

There were five dietary treatments (Table 2). Diet 1 was a NC-whey protein-blood cell diet containing 0.15% available P and 0.55% Ca. Diet 2 was a LPC-whey protein-blood cell diet containing 0.24% available P and 0.55% Ca. Diet 3 was a NC-whey protein-blood cell-inorganic P diet, which was similar to Diet 1, but with monosodium phosphate added to increase the available P to 0.24% to equal the concentration of available P in Diet 2. Diet 4 was a NC-soybean meal diet containing 1.17% dicalcium phosphate, 0.30% available P, and 0.65% Ca. Diet 5 was a LPC-soybean meal diet containing 0.65% dicalcium phosphate, 0.30% available P, and 0.65% Ca.

Diets 1, 2, and 3 were semipurified diets in which the corn hybrids were the only source of phytate. Diets 1 and 2 were not supplemented with any inorganic P. Thus, the concentrations of available P in Diets 1 to 3 were below the NRC (1998) requirement, which provided a strong test of the ingredients as sources of available P. Dietary Ca was also reduced to minimize the negative effects of Ca on P utilization (Liu et al., 1998a).

Measurements. Diets contained 0.05% chromic oxide as a nondigestible indicator. Fecal grab samples and total urine collections were made twice daily from d 29 to 33. Fecal samples were immediately frozen in plastic freezer bags until analyzed. Urine was collected in plastic pails containing 25 mL of 6 *N* HCl. Total urine volume was recorded, and 10% was saved in 1-L screw-cap plastic bottles that were immediately frozen until analyzed. The 5-d fecal collections for individual pigs were thawed, pooled, and air-dried at 55°C. The dried fecal samples and samples of each diet were ground to pass through a 1.0-mm screen before analysis in triplicate for N, Ca, and P as described for the dietary ingredients. The 5-d urine collections for individual pigs were thawed, pooled, mixed, and subsampled before analysis for N, Ca, and P. Subsamples of feces, diet, and urine were also analyzed in triplicate for Cr (AOAC, 1990) using the atomic absorption spectrophotometry procedure described for Ca, and for GE by oxygen bomb calorimeter (Parr Instrument Co., Moline, IL).

On d 35, the pigs were killed (electrically stunned followed by exsanguination). The right-front foot and elbow were removed and refrigerated at 2°C until the third metacarpal and radius bones had been removed and cleaned of all adhering soft tissue, within 72 h, for size measurements and for the determination of breaking strength and ash weight. A caliper (Model CDS6, Mitutoyo Corp., Japan) was used to measure metacarpal length and the midpoint widths at the narrowest and widest points. Breaking strength of the fresh bones was determined using an Instron testing machine (Model TML, Instron Corp., Canton, MA), similar to the procedure described by Crenshaw (1986). Force was applied to the center of the bone, which was held by two supports spaced 3.0 cm apart. After the determination of breaking strength, the bones were wrapped with cheesecloth, boiled in deionized water for 2 h, dried at 55°C for 24 h, and extracted with ethyl ether for 4 to 5 d. Ash weight was determined after the fat-free bones were dried at 55°C and 100°C for 18 and 2 h, respectively, and ashed in a muffle furnace at 600°C for 16 h.

In Vitro Phosphorus Release Comparison

Both NC and LPC samples were ground through a 1-mm screen and refrigerated until analyzed. The in vitro digestion procedure that was developed to simulate the digestive system of growing swine for the purpose of predicting phosphorus release from corn-soybean meal diets and feed ingredients was used (Liu et

Table 2. Ingredient composition (%) of air-dry diets

Item	Dietary treatment[a]				
Treatment No.:	1	2	3	4	5
Treatment description:	NC-WP-BC	LPC-WP-BC	NC-WP-BC-iP	NC-SBM	LPC-SBM
Normal corn	72.00	—	72.00	74.20	—
Mutant corn	—	72.00	—	—	74.60
Whey protein concentrate	19.00	19.00	19.00	—	—
Soybean meal, 48%	—	—	—	20.00	20.00
Spray-dried blood cells	2.00	2.00	2.00	—	—
Lard	2.87	2.83	2.89	2.50	2.36
Monosodium phosphate[b]	—	—	0.37	—	—
Dicalcium phosphate[c]	—	—	—	1.17	0.65
Limestone	0.95	0.95	0.95	0.73	1.02
Lactose	0.80	0.80	0.80	—	—
L-Glutamic acid	0.75	0.84	0.69	—	—
Cellulose	0.37	0.34	0.04	—	—
White salt	0.40	0.40	0.40	0.40	0.40
Vitamin & medication premixes[d]	0.45	0.45	0.45	0.45	0.45
Trace mineral premix[e]	0.15	0.15	0.15	0.15	0.15
L-Lysine HCl	0.15	0.15	0.15	0.30	0.28
DL-Methionine	0.06	0.04	0.06	0.05	0.04
Chromic oxide	0.05	0.05	0.05	0.05	0.05
Dietary composition[f]					
Ca	0.55	0.55	0.55	0.65	0.65
Total P	0.29	0.30	0.38	0.54	0.45
Phytic acid P	0.14	0.06	0.14	0.24	0.15
Available P[g]	0.15	0.24	0.24	0.30	0.30
CP	15.30	15.30	15.30	16.37	16.23
Lysine	1.05	1.05	1.05	1.00	1.00
Methionine + cystine	0.65	0.64	0.65	0.60	0.61
Tryptophan	0.22	0.21	0.22	0.19	0.19
ME, Mcal/kg	3.55	3.55	3.55	3.42	3.42

[a]NC = normal corn, LPC = low-phytate mutant corn, WP = whey protein concentrate, BC = spray-dried blood cells, iP = inorganic P, and SBM = soybean meal.
[b]Monosodium phosphate contains 25.3% P.
[c]Dicalcium phosphate contains 22.1% Ca and 18.5% P.
[d]Vitamin and medication premixes provided per kilogram of diet: 11,000 IU of vitamin A acetate; 1,100 IU of vitamin D_3; 44.1 IU of vitamin E as DL-α-tocopheryl acetate; 4.0 mg of vitamin K as menadione sodium dimethylpyrimidinol bisulfite complex; 30.3 μg of vitamin B_{12}; 8.3 mg of riboflavin; 28.1 mg of pantothenic acid as D-calcium pantothenate; 33.1 mg of niacin; 551.3 mg of choline as choline chloride; 220.5 μg of biotin; 1.65 mg of folic acid; and 110 mg of tylosin.
[e]Trace mineral premix provided per kilogram of diet: 165 mg of Zn as $ZnSO_4$; 165 mg of Fe as $FeSO_4$; 33 mg of Mn as Mn SO_4; 16.5 mg of Cu as $CuSO_4$; 0.3 mg of I as $Ca(IO_3)_2$; and 0.3 mg of Se as $Na_2Se\ O_3$.
[f]Composition is based on triplicate analyses of each diet except for ME that was calculated.
[g]Available P is calculated by subtracting phytic acid P from total P.

Table 3. Pig growth performance

Item	Treatment No.:	1	2	3	4	5	
		Dietary treatment[a]					
	Treatment description:	NC-WP-BC	LPC-WP-BC	NC-WP-BC-iP	NC-SBM	LPC-SBM	
	Available P, %:	0.15	0.24	0.24	0.30	0.30	SEM
Pig wt., kg							
d 0		14.51	14.51	14.51	14.52	14.51	0.10
d 35[b]		36.55	40.36	41.08	41.16	41.97	0.96
ADG, g[b]		630	738	759	761	784	27
ADFI, g[c]		1,356	1,435	1,445	1,481	1,500	35
Gain/feed, g/kg[b]		464	515	526	512	523	14

[a]NC = normal corn, LPC = low-phytate mutant corn, WP = whey protein concentrate, BC = spray-dried blood cells, iP = inorganic P, and SBM = soybean meal.
[b]Treatment 1 vs Treatment 2 and Treatment 1 vs Treatment 3 ($P < 0.01$).
[c]Treatment 1 vs Treatment 2 ($P < 0.08$).

al., 1997b; 1998b). The in vitro procedure was replicated three times for estimation of P release from NC and LPC. Phosphorus concentration in the supernate was determined colorimetrically by the molybdovanadate method (AOAC, 1990). The availability of P was estimated to be 56% for LPC and 11% for NC.

Statistical Analysis. Data from all five treatments were analyzed as a randomized complete block design with individual pigs as the experimental units (Snedecor and Cochran, 1989) using the statistical procedures of SAS (SAS Inst. Inc., Cary, NC). The preplanned single-df comparisons were treatments 1 vs 2, 1 vs 3, 2 vs 3, and 4 vs 5.

Results

In Vivo Experiment

Pig Growth Performance. Pigs fed semipurified LPC Diet 2 gained 17% more ($P < 0.01$) BW, tended ($P < 0.08$) to consume more feed (6%), and were 11% more efficient ($P < 0.01$) in feed conversion (gain/feed) compared with pigs fed semipurified NC Diet 1 (Table 3). Pigs fed LPC Diet 2 had similar ($P > 0.6$) ADFI, ADG and gain/feed ratios compared with pigs fed NC Diet 3 containing monosodium phosphate to equalize the available P concentration to that in LPC Diet 2. Pigs fed the practical NC-soybean meal Diet 4 or the practical LPC-soybean meal Diet 5, which were formulated to contain equal concentrations of available P, were also similar ($P > 0.6$) in growth performance criteria (Table 3).

Bone Characteristics. For metacarpal bone characteristics (Table 4), pigs fed LPC Diet 2 had higher fat-free dry weight ($P < 0.001$), ash weight ($P < 0.001$), and breaking strength ($P < 0.01$), and they tended ($P < 0.07$) to have higher fresh bone weight, compared with pigs fed NC Diet 1. There was a trend ($P < 0.09$) for pigs fed NC Diet 3 to have a higher metacarpal fat-free dry weight, ash weight, and breaking strength compared with pigs fed LPC Diet 2. The only metacarpal criterion that differed for pigs fed the practical diets was that pigs fed practical LPC Diet 5 had a higher ($P < 0.01$) metacarpal breaking strength compared with pigs fed practical NC Diet 4.

For radius bone characteristics (Table 4), pigs fed NC Diet 1 had lower fresh bone weight ($P < 0.01$), fat-free dry weight ($P < 0.001$), ash weight ($P < 0.001$), and breaking strength ($P < 0.01$) than pigs fed Diet 2 or Diet 3, which did not differ ($P > 0.3$) in radius characteristics. Pigs fed practical LPC Diet 5 had a higher ($P < 0.05$) radius fat-free dry weight, ash weight, and breaking strength than pigs fed practical NC Diet 4.

For metacarpal and radius bone measurements, there were no preplanned single-df treatment comparison differences ($P \geq 0.2$) for bone length or midpoint widths at the narrowest and widest points for either bone (data not presented).

Phosphorus and Ca Utilization. With diets that contained equal concentrations of available P, grams of total P consumed per day were higher ($P < 0.001$) for pigs fed NC Diet 3 or NC Diet 4 than for pigs fed LPC Diet 2 or LPC Diet 5, respectively (Table 5). Pigs fed LPC Diet 2 or LPC Diet 5 excreted less ($P < 0.001$) fecal P (g/d) than pigs fed NC Diet 3 or NC Diet 4, respectively. Pigs fed NC Diet 3 tended ($P < 0.07$) to excrete more grams of urinary P per day than pigs fed LPC Diet 2. Pigs fed NC Diet 1 absorbed and retained fewer ($P < 0.001$) grams of P per day than pigs fed LPC Diet 2 or NC Diet 3 which were not different ($P > 0.9$). Expressed as a percentage of intake, P absorption and retention were higher ($P < 0.001$) for pigs fed LPC Diet 2 compared with pigs fed NC Diet 1 or NC Diet 3. Phosphorus absorption and retention (g/d and percentage of intake) were similar ($P \geq 0.2$ to 0.6) for pigs fed practical NC Diet 4 or practical LPC Diet 5, whereas fecal P excretion was higher ($P < 0.001$) for pigs fed NC Diet 4 compared with pigs fed LPC Diet 5.

Calcium intake per day was not different for Treatments 1 to 3 ($P > 0.3$) or Treatment 4 vs Treatment 5 ($P > 0.8$) (Table 5). Fecal excretion of Ca (g/d) was lower ($P < 0.01$) for pigs fed LPC Diet 2 compared with pigs fed NC Diet 1 or NC Diet 3. Urinary excretion of Ca was higher ($P < 0.01$) for pigs fed NC Diet 1 compared

Table 4. Bone characteristics

Item	Treatment No.: Treatment description: Available P, %:	Dietary treatment[a] 1 NC-WP-BC 0.15	2 LPC-WP-BC 0.24	3 NC-WP-BC-iP 0.24	4 NC-SBM 0.30	5 LPC-SBM 0.30	SEM
Metacarpal bone							
Fresh wt, g[b]		13.60	14.97	14.73	14.77	14.96	0.51
Fat-free dry wt, g[c]		3.36	4.11	4.35	4.37	4.61	0.12
Ash wt, g[c]		1.82	2.33	2.50	2.62	2.74	0.07
Breaking strength, kg[d]		36.91	48.00	54.99	51.75	62.06	2.47
Radius bone							
Fresh wt, g[e]		39.38	44.99	44.02	43.64	44.88	1.11
Fat-free dry wt., g[f]		11.73	14.37	15.12	15.01	16.47	0.47
Ash wt., g[f]		5.42	7.39	7.73	8.13	8.89	0.23
Breaking strength, kg[g]		64.62	86.70	94.37	99.66	115.43	5.70

NC = normal corn, LPC = low-phytate mutant corn, WP = whey protein concentrate, BC = spray-dried blood cells, iP = inorganic P, and SBM = soybean meal.
[b]Treatment 1 vs Treatment 2 ($P < 0.07$).
[c]Treatment 1 vs Treatment 2, and Treatment 1 vs Treatment 3 ($P < 0.001$). Treatment 2 vs Treatment 3 ($P < 0.09$).
[d]Treatment 1 vs Treatment 2, and Treatment 1 vs Treatment 3 ($P < 0.01$). Treatment 2 vs Treatment 3 ($P < 0.09$), and Treatment 4 vs Treatment 5 ($P < 0.01$).
[e]Treatment 1 vs Treatment 2, and Treatment 1 vs Treatment 3 ($P < 0.01$).
[f]Treatment 1 vs Treatment 2, and Treatment 1 vs Treatment 3 ($P < 0.001$). Treatment 4 vs Treatment 5 ($P < 0.05$).
[g]Treatment 1 vs Treatment 2, and Treatment 1 vs Treatment 3 ($P < 0.01$). Treatment 4 vs Treatment 5 ($P < 0.05$).

with pigs fed LPC Diet 2 or NC Diet 3. Calcium absorption in grams per day or as a percentage of intake was lower ($P < 0.05$) for pigs fed NC Diet 1 than for pigs fed LPC Diet 2 or NC Diet 3, which did not differ ($P > 0.8$). Retention of Ca (g/d or percentage of intake) was also lower ($P < 0.001$) for pigs fed NC Diet 1 than for pigs fed LPC Diet 2 or NC Diet 3, which did not differ ($P > 0.4$). Calcium absorption and retention (g/d and percent-

Table 5. Phosphorus and calcium utilization

Item	Treatment No.: Treatment description: Available P, %:	Dietary treatment[a] 1 NC-WP-BC 0.15	2 LPC-WP-BC 0.24	3 NC-WP-BC-iP 0.24	4 NC-SBM 0.30	5 LPC-SBM 0.30	SEM
ADFI, d 28 to 33, kg		2.02	2.08	2.13	2.32	2.34	0.06
Phosphorus							
Intake, g/d[b]		5.67	6.03	8.10	12.50	10.51	0.25
Fecal, g/d[c]		3.55	2.01	4.00	7.41	5.92	0.28
Urinary, g/d[d]		0.10	0.10	0.22	0.20	0.29	0.04
Absorbed, g/d[e]		2.12	4.02	4.10	5.09	4.59	0.37
Retained, g/d[e]		2.02	3.92	3.89	4.89	4.30	0.34
Absorbed/intake, %[f]		37.32	66.74	50.62	40.27	43.50	2.89
Retained/intake, %[f]		35.57	65.06	47.99	38.74	40.76	2.71
Calcium							
Intake, g/d		11.13	11.39	11.73	15.05	15.18	0.37
Fecal, g/d[g]		3.65	2.39	3.31	6.38	6.26	0.29
Urinary, g/d[h]		3.93	3.03	2.47	1.02	1.78	0.21
Absorbed, g/d[i]		7.48	8.51	8.42	8.68	8.92	0.33
Retained, g/d[j]		3.56	5.98	5.95	7.66	7.15	0.40
Absorbed/intake, %[i]		67.21	74.72	71.82	57.49	58.81	1.90
Retained/intake, %[j]		31.74	52.50	50.68	50.65	46.94	2.33

[a]NC = normal corn, LPC = low-phytate mutant corn, WP = whey protein concentrate, BC = spray-dried blood cells, iP = inorganic P, and SBM = soybean meal.
[b]Treatment 1 vs Treatment 3, Treatment 2 vs Treatment 3, and Treatment 4 vs Treatment 5 ($P < 0.001$).
[c]Treatment 1 vs Treatment 2, Treatment 2 vs Treatment 3, and Treatment 4 vs Treatment 5 ($P < 0.001$).
[d]Treatment 1 vs Treatment 3, and Treatment 2 vs Treatment 3 ($P < 0.07$).
[e]Treatment 1 vs Treatment 2, and Treatment 1 vs Treatment 3 ($P < 0.001$).
[f]Treatment 1 vs Treatment 2, and Treatment 2 vs Treatment 3 ($P < 0.001$). Treatment 1 vs Treatment 3 ($P < 0.01$).
[g]Treatment 1 vs Treatment 2, and Treatment 2 vs Treatment 3 ($P < 0.01$).
[h]Treatment 1 vs Treatment 2, and Treatment 1 vs Treatment 3 ($P < 0.01$). Treatment 4 vs Treatment 5 ($P < 0.02$).
[i]Treatment 1 vs Treatment 2, and Treatment 1 vs Treatment 3 ($P < 0.05$).
[j]Treatment 1 vs Treatment 2, and Treatment 1 vs Treatment 3 ($P < 0.001$).

Table 6. Nitrogen and energy utilization

Item	Treatment No.: Treatment description: Available P, %:	Dietary treatment[a] 1 NC-WP-BC 0.15	2 LPC-WP-BC 0.24	3 NC-WP-BC-iP 0.24	4 NC-SBM 0.30	5 LPC-SBM 0.30	SEM
Nitrogen							
Intake, g/d[b]		46.61	52.62	50.55	60.67	64.05	1.60
Fecal, g/d		7.17	7.91	8.72	12.56	14.12	0.96
Urinary, g/d		9.35	7.96	8.18	9.05	9.96	1.13
Absorbed, g/d[c]		39.44	44.72	41.82	48.11	49.94	1.35
Retained, g/d[d]		30.09	36.76	33.64	39.06	39.97	1.06
Absorbed/intake, %		84.63	85.04	82.72	79.41	77.98	1.40
Retained/intake, %[e]		64.74	69.98	66.60	64.77	62.37	1.99
Energy							
Intake, kcal GE/d[f]		9,330	10,452	10,476	10,765	11,252	305
Fecal, kcal/d		924	1,032	1,071	1,513	1,745	98
Urinary, kcal/d		139	139	146	132	118	14
Absorbed (DE), kcal/d[f]		8,406	9,420	9,405	9,252	9,506	259
Retained (ME), kcal/d[f]		8,267	9,282	9,259	9,119	9,389	253
Absorbed/intake, %		90.12	90.15	89.78	85.97	84.50	0.76
Retained/intake, %		88.64	88.83	88.38	84.75	83.45	0.73

[a]NC = normal corn, LPC = low-phytate mutant corn, WP = whey protein concentrate, BC = spray-dried blood cells, iP = inorganic P, and SBM = soybean meal.
[b]Treatment 1 vs Treatment 2 ($P < 0.01$. Treatment 1 vs Treatment 3 ($P < 0.09$).
[c]Treatment 1 vs Treatment 2 ($P < 0.01$).
[d]Treatment 1 vs Treatment 2 ($P < 0.01$). Treatment 1 vs Treatment 3, and Treatment 2 vs Treatment 3 ($P < 0.05$).
[e]Treatment 1 vs Treatment 2 ($P < 0.07$).
[f]Treatment 1 vs Treatment 2, and Treatment 1 vs Treatment 3 ($P < 0.01$).

age of intake) were similar ($P \geq 0.3$) for pigs fed practical NC Diet 4 and those fed practical LPC Diet 5.

Nitrogen and Energy Utilization. Nitrogen intake was lower ($P < 0.01$) for pigs fed NC Diet 1 than for pigs fed LPC Diet 2, and tended ($P < 0.09$) to be lower for pigs fed NC Diet 1 than for pigs fed NC Diet 3 (Table 6). The grams of N absorbed and retained per day were also lower ($P < 0.01$) for pigs fed NC Diet 1 than for pigs fed LPC Diet 2. Pigs fed NC Diet 1 tended ($P < 0.07$) to have lower percentage of N intake retained than those fed LPC Diet 2. Nitrogen retention (g/d) was higher ($P < 0.05$) for pigs fed LPC Diet 2 than for pigs fed NC Diet 3 containing the same concentration of available P, and for pigs fed NC Diet 3 than for pigs fed NC Diet 1.

Gross energy intake, kilocalories absorbed per day (apparent DE), and kilocalories retained per day (apparent ME) were lower ($P < 0.01$) for pigs fed NC Diet 1 than for pigs fed LPC Diet 2 or NC Diet 3 but were not different ($P > 0.9$) between pigs fed LPC Diet 2 or NC Diet 3 (Table 6). The lower GE, DE, and ME intakes by pigs fed NC Diet 1 occurred because of the lower ADFI by pigs fed NC Diet 1 compared with pigs fed LPC Diet 2 or NC Diet 3 (Table 3). This theory is supported by the fact that apparent energy absorption and retention expressed as percentages of intake were not different ($P > 0.7$) for pigs fed Diets 1, 2, or 3. For pigs fed practical NC Diet 4 or practical LPC Diet 5, there were no differences ($P < 0.4$) between these two diets for any N or energy balance criteria measured.

Discussion

These in vivo and in vitro experiments confirm the higher availability of P in mutant LPC compared with the nearly isogenic NC previously reported with broiler chicks (Li et al., 2000). Using our in vitro procedure (Liu et al., 1997b, 1998b), our P availability estimates of 56% for LPC and 11% for NC are nearly identical to previously reported in vitro values for these corn hybrids (Spencer et al., 2000). This is a fivefold increase in available P for LPC compared with the isogenic NC.

Our semipurified Diets 1, 2, and 3, in which corn was the only source of phytic acid, were designed to address our first in vivo objective of confirming the differences in available P between LPC and NC. Because the source of corn was the only difference between Diets 1 and 2, treatment differences may be attributed to the higher available P and lower phytic acid content of LPC vs NC. Diet 3 was Diet 1 supplemented with inorganic P to match the higher level of available P in Diet 2, so the only difference between Diets 2 and 3 was the level of phytic acid. The comparison of Diets 2 and 3 would determine whether the decrease in phytic acid concentration in LPC would affect the nutritional value of LPC. Diets 4 and 5 were practical corn-soybean meal diets formulated to meet minimum NRC (1998) requirements. Both diets contained 0.30% available P, with the LPC in Diet 5 providing a greater proportion of the available P compared with the NC in Diet 4.

The greater growth performance, bone breaking strength, apparent P absorption and retention, apparent Ca absorption and retention, and apparent N retention observed in pigs fed LPC Diet 2 (compared with NC Diet 1) confirmed the greater availability of P in LPC. Dietary Ca was lowered 0.1% to 0.55% in the semipurified diets to keep the Ca:total P ratio below 2, because it is well known that excessive Ca reduces P utilization (Nahapetian and Young, 1980; Liu et al., 1998a, 2000). Semipurified NC Diet 3 was supplemented with inorganic P to contain 0.24% available P, the same level of available P provided by LPC Diet 2. Results showed that pigs fed Diets 2 or 3 were similar in most of the measurements made for comparison of LPC and NC, indicating that the estimated available P levels determined for the corn hybrids were reasonable, and that lowering the phytic acid content in LPC did not compromise the nutritional value of LPC.

The NC Diet 4 and LPC Diet 5 were practical corn-soybean meal diets supplemented with dicalcium phosphate to contain the same adequate (NRC, 1998) levels of available P and Ca to determine whether using LPC in commercial diets would affect growing pig performance. Our results showed that pigs fed these diets were similar in growth performance, apparent P and Ca utilization, apparent N utilization, and apparent DE and ME utilization. Thus, these results indicate that growth performance, P and Ca utilization, and energy utilization were similar for pigs fed diets containing either LPC or NC regardless of whether corn was the only source of phytic acid or whether soybean meal was used as a protein supplement when the NC diets contained adequate inorganic P to equal the available P in the LPC diets (Diet 2 vs 3, and Diet 4 vs 5). Nitrogen utilization was also similar when both corn hybrids were supplemented with soybean meal. The lack of differences between the two corn hybrids in N and energy utilization when soybean meal was the protein supplement is in agreement with the report by Spencer et al. (2000).

However, pigs fed LPC Diet 5 had greater bone breaking strength and ash weight than pigs fed NC Diet 4 that contained the same estimated level of available P. This suggests that we may have slightly underestimated the available P concentration in LPC, or that the lower phytic acid concentration in LPC resulted in greater utilization of available P in LPC compared with NC. This experiment demonstrated that total P and phytic acid P concentrations may be used to provide a useful estimate of the available P concentration in corn fed to swine. This information has practical application in estimating available P values in corn for use in diet formulation. Research evaluating the use of the enzyme phytase in low-P corn-soybean meal diets fed to growing and finishing pigs showed that the addition of 500 phytase units/kg of diet increased growth performance and P absorption equal to that of pigs fed the positive control diets (Liu et al., 1998a). However, bone breaking strength was less for pigs fed the phytase diets compared with pigs fed the positive control diets, suggesting that bone strength was compromised when using 500 phytase units/kg diet (Liu et al., 1998a). Another practical limitation to the use of the enzyme phytase in animal feeds is that the thermal stress of pelletizing or extrusion may deactivate the enzyme (Guenther, 1996).

The most significant effect of feeding LPC was the reduction in P excretion. For pigs fed the semipurified diets containing the same level of available P (LPC Diet 2 and NC Diet 3), P excretion (g/d, feces + urine) was reduced 50% by LPC compared with NC (2.11 vs 4.22 g/d, respectively). For pigs fed the practical corn-soybean meal diets (LPC Diet 5 and NC Diet 4), P excretion was reduced a less dramatic 18.4% by LPC compared with NC (6.21 vs 7.61 g/d). Thus, the phytic acid in soybean meal has a significant negative effect on P utilization in a corn-soybean meal diet. Supplementation of a low-P corn-soybean meal diet with 250 phytase units/kg reduced fecal P excretion 48% when the diet was soaked before feeding, and 37% by feeding the diet as a dry meal, compared with pigs fed a dry positive control diet (Liu et al., 1997a). Therefore, the negative effect of soybean meal on P utilization in a low-phytic acid grain-soybean meal diet may be reduced by supplementation of these diets with phytase. In theory, phytase will increase the available P, reducing the inorganic P supplementation required and further reduce P excretion in swine waste. However, the development of a low-phytic acid soybean meal to use in combination with a low-phytic acid grain will reduce or eliminate the need for phytase supplementation of swine diets. The results of this experiment and those of Spencer et al. (2000) provide additional evidence to support the validity of our in vitro procedure to estimate the availability of P in plant-based diets and feed ingredients for growing swine (Liu et al., 1997b; 1998b).

In conclusion, the P in LPC was about fivefold more available than the P in NC as a result of the reduction in phytic acid in LPC. The reduction in phytic acid did not compromise the nutritional value of LPC, and P excretion was significantly reduced by feeding LPC. Our in vitro procedure provided a valid estimate for the availability of P in both corn hybrids.

Implications

Formulation of swine diets with low-phytate corn containing the *lpa1-1* allele will assist the swine industry in becoming more environmentally friendly by greatly reducing the excretion of phosphorus in swine waste. Bone strength was increased when low-phytate corn replaced normal corn with soybean meal as the protein supplement. Lowering the phytate concentration did not have any adverse nutritional effects on low-phytate corn.

Literature Cited

AOAC. 1990. Official Methods of Analysis. 15th ed. Association of Official Analytical Chemists, Arlington, VA.

Benson, J. V., Jr., and J. A. Patterson. 1971. Chromatographic advances in amino acid and peptide analysis using spherical resins and their applications in biochemistry and medicine. In: A. Niederweiser and G. Pataki (ed.) New Techniques in Amino Acid, Peptide and Protein Analysis. pp 1–73. Ann Arbor Sci. Publ., Ann Arbor, MI.

Crenshaw, T. D. 1986. Reliability of dietary Ca and P levels and bone mineral content as predictors of bone mechanical properties at various time period in growing swine. J. Nutr. 116:2155–2170.

Cromwell, G. L., R. D. Coffey, G. R. Parker, H. J. Monegue, and J. H. Randolph. 1995. Efficacy of a recombinant-derived phytase in improving the bioavailability of phosphorus in corn-soybean meal diets for pigs. J. Anim. Sci. 73:2000–2008.

Cromwell, G. L., T. S. Stahly, R. D. Coffey, H. J. Monegue, and J. H. Randolph. 1993. Efficacy of phytase in improving the bioavailability of phosphorus in soybean meal and corn-soybean meal diets for pigs. J. Anim. Sci. 71:1831–1840.

Ertl, D. S., K. A. Young, and V. Raboy. 1998. Plant genetic approaches to phosphorus management in agricultural production. J. Environ. Qual. 27:299–304.

Guenther, C. 1996. Stability of Natuphos® phytase through feed processing. In: M. B. Coelho and E. T. Kornegay (ed.) Phytase in Animal Nutrition and Waste Management. pp 655–666. DC 9601. BASF Corp., Mount Olive, NJ.

Heinzl, W. 1996. Natuphos® technical specifications. In: M. B. Coelho and E. T. Kornegay (ed.) Phytase in Animal Nutrition and Waste Management. pp 693–700. DC 9601, BASF Corp., Mount Olive, NJ.

Li, Y. C., D. R. Ledoux, T. L. Veum, V. Raboy, and D. S. Ertl. 2000. Effects of low phytic acid corn on phosphorus utilization, performance, and bone mineralization in broiler chicks. Poult. Sci. 79:1444–1450.

Liu, J., D. W. Bollinger, D. R. Ledoux, M. R. Ellersieck, and T. L. Veum. 1997a. Soaking increases the efficacy of supplemental microbial phytase in a low-phosphorus corn-soybean meal diet for growing pigs. J. Anim. Sci. 75:1292–1298.

Liu, J., D. W. Bollinger, D. R. Ledoux, and T. L. Veum. 1998a. Lowering the dietary calcium to total phosphorus ratio increases phosphorus utilization in low-phosphorus corn-soybean meal diets supplemented with microbial phytase for growing-finishing pigs. J. Anim. Sci. 76:808–813.

Liu, J., D. W. Bollinger, D. R. Ledoux, and T. L. Veum. 2000. Effects of dietary calcium:phosphorus ratios on apparent absorption of calcium and phosphorus in the small intestine, cecum and colon of pigs. J. Anim. Sci. 78:106–109.

Liu, J., D. R. Ledoux, and T. L. Veum. 1997b. In vitro procedure for predicting the enzymatic dephosphorylation of phytate in corn-soybean meal diets for growing swine. J. Agric. Food Chem. 45:2612–2617.

Liu, J., D. R. Ledoux, and T. L. Veum. 1998b. In vitro prediction of phosphorus availability in feed ingredients for swine. J. Agric. Food Chem. 46:2678–2681.

Lott, J. N. A., I. Ockenden, V. Raboy, and G. D. Batten. 2000. Phytic acid and phosphorus in crop seeds and fruits:a global estimate. Seed Sci. Res. 10:11–33.

Maga, J. A. 1982. Phytate: Its chemistry, occurrence, food interactions, nutritional significance, and methods of analysis. J. Agric. Food Chem. 30:1–9.

Nahapetian, A., and V. R. Young. 1980. Metabolism of ^{14}C-phytate in rats: Effect of low and high dietary calcium intake. J. Nutr. 110:1458–1472.

NRC. 1998. Nutrient Requirements of Swine. 10th ed. National Academy Press, Washington, DC.

Pointillart, A., N. Fontaine, and M. Thomasset. 1984. Phytate phosphorus utilization and intestinal phytases in pigs fed low phosphorus:wheat or corn diets. Nutr. Rep. Int. 29:473–483.

Pointillart, A., A. Fourdin, and N. Fontaine. 1987. Importance of cereal phytase activity for phytate phosphorus utilization by growing pigs fed diets containing triticale or corn. J. Nutr. 117:907–913.

Raboy, V., P. F. Gerbasi, K. A. Young, S. D. Stoneberg, S. G. Pickett, A. T. Bauman, P. P. N. Murthy, W. F. Sheridan, and D. S. Ertl. 2000. Origin and seed phenotype of maize low phytic acid 1-1 and low phytic acid 2-1. Plant Physiol. 124:355–368.

Snedecor, G. W., and W. G. Cochran. 1989. Statistical Methods. 8th ed. Iowa State University Press, Ames.

Simons, P. C. M., H. A. J. Versteegh, A. W. Jongbloed, P. A. Kemme, P. Slump, K. D. Bos, M. G. E. Wolters, R. F. Beudeker, and G. J. Verchoor. 1990. Improvements of phosphorus availability by microbial phytase in broilers and pigs. Br. J. Nutr. 64:525–540.

Spencer, J. D., G. L. Allee, and T. E. Sauber. 2000. Phosphorus bioavailability and digestibility of normal and genetically modified low-phytate corn for pigs. J. Anim. Sci. 78:675–681.

Spies, J. R., and D. C. Chambers. 1949. Chemical determination of tryptophan in proteins. Procedure N. Anal. Chem. 21:1249–1266.

Sugiura, S. H., V. Raboy, K. A. Young, F. M. Dong, and R. W. Hardy. 1999. Availability of phosphorus and trace minerals in low-phytate varieties of barley and corn for rainbow trout (Oncorhynchus mykiss). Aquaculture 170:285–296.